3.2.1.2.2 Lamina propria

Collagen-fibres: The lamina mucosa consisted of a dense connective tissue, mainly collagenous fibres. There was no true muscularis mucosa separating the mucosa from submucosa. However, the deep region of the mucosa showed a condensation of connective tissue which extended into the core of the papillae (Fig. 6). This condensation of the connective tissue, contained collagenous fibres (Fig. 22) and α-actin smooth muscle fibres (see immunohistochemistry-chapter). The sub-epithelial connective tissue showed many epithelial projections (papillary body), which were well developed at the papillae (especially at the apex) than at the interpapillary regions (Fig. 6 and 8).

The amount of the collagenous fibres at the core of the papillae was significantly increased in concentrate-fed group (Fig. 22). This increase in the amount of collagenous fibres at the core of papillae is correlated to the duration of concentrate feeding and the size of papillae (Fig.22 a, b, c, d, e, f, g and h). In 12 weeks concentrate-fed group, papillae showed branching of the lamina propria giving off secondary papillae (Fig. 22 g).

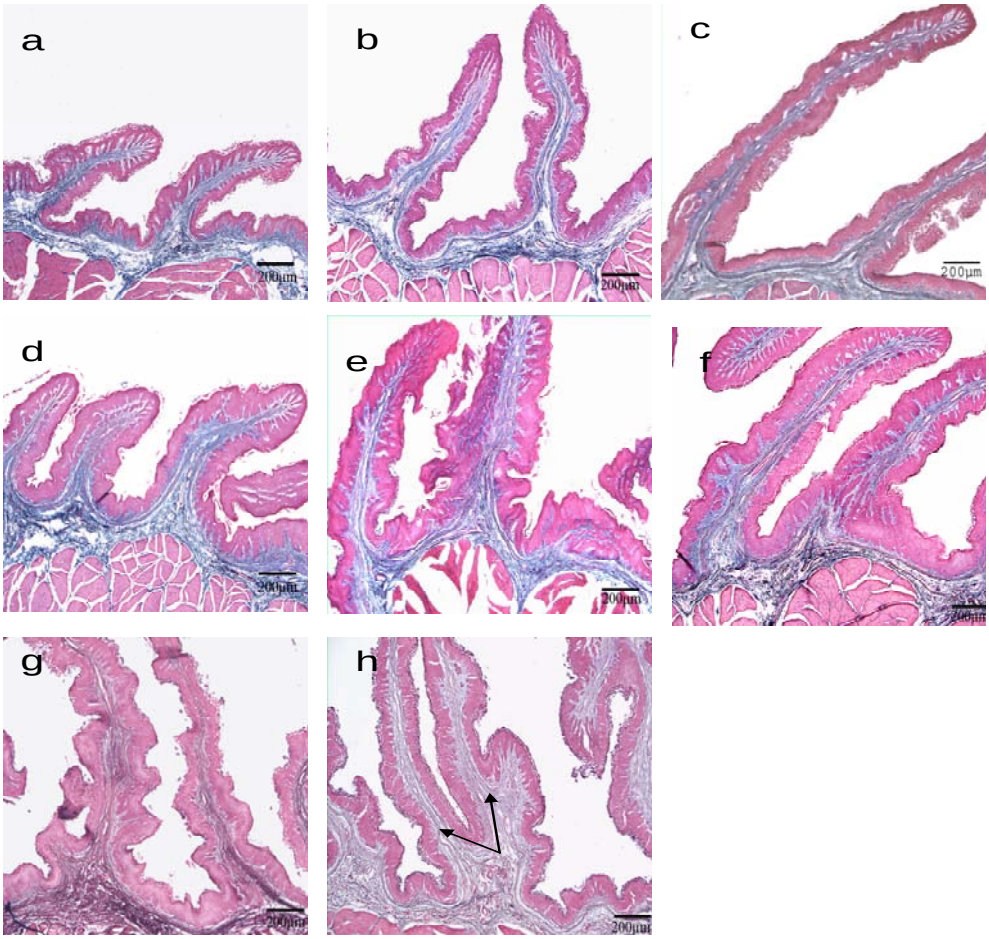

Fig. 22: Sections of papillae from eight concentrate-fed groups illustrate increasing amounts of collagenous fibers (blue colour) with extention of concentrate feeding. a. Hay-fed group b. 2 days concentrate-fed group c. 4 days concentrate-fed group d.1 week concentrate-fed group e. 2 weeks concentrate-fed group f. 4 weeks concentrate-fed group g. 6 weeks concentrate-fed group h. 12 weeks concentrate-fed group. Arrow (→) pointed to secondary papillae. (H and E) (Scale 200µm)

Blood vessels: Large blood vessels were observed in the connective tissue of the lamina-submucosa. They branched at the base of the papillae into arterioles, which extended into the core of the papillae. These small vessels continued as capillaries in the connective tissue present between the epithelial pegs (papillary body). The density of blood vessels and capillaries moderately increased from the 4th day of concentrate feeding (Fig. 16 a), and highly increased from the 4th week of concentrate feeding. In papillary body, sinusoid-like capillaries were observed (Fig. 19 a and 21 g).

Lymphocytes: A few lymphocytes were observed scattered in the connective tissues of the core of the papillae of all groups. However, in 6 and 12 weeks concentrate-fed groups,

lymphocytes were observed, especially at the lamina propria adjacent to the stratum basale (Fig. 21 c, h).

3.2.1.3 Ruminal mucosa-carbohydrates content (Periodic Acid-Schiff Reaction = PAS)

Results of the PAS reaction, which was applied to the rumen- tissues isolated from the sheep of all groups, are shown in figures 23, 24, 25, 26, 27 and table 10.

Periodic Acid-Schiff-positive, Diastase-Resistant Material: The PAS reaction was weak in sheep fed either hay ad libitum (control) or hay ad libitum and concentrate-diet for 2 days or 4 days. This reaction was observed in both stratum corneum and granulosum. However, stratum spinosum and stratum basale were PAS-negative (Figs. 23 a, 24 a, b and table 10). The intense staining at the cell boundaries of the stratum corneum was the most striking feature of the epithelium in PAS preparations. The intercellular substance showed also a PAS-positive. There was also a light, diffuse intra-cellular PAS reaction of the superficial horn cells blended with the intense reaction of the ingesta and bacteria on the surface of the epithelium. The nuclei did not show PAS reaction in normal epithelium, while the cell membranes were weakly stained. The PAS reaction in the stratum corneum and granulosum in 1-2 weeks concentrate-fed groups followed the same pattern of the previous concentrate-fed groups. However, the degree of PAS- reaction was moderate (Fig. 23 b). Furthermore, in the stratum granulosum, PAS-positive, diastase-resistant droplets were stained, but they were smaller than the keratohyalin granules seen in sections stained with haematoxylin and eosin. The superficial cells of stratum spinosum showed weak PAS-reaction. Both stratum spinosum (deep layer) and basale showed a PAS-negative reaction.

In 4-6 weeks concentrate-fed groups, the degree of PAS- reaction was strong (Fig. 23, 25 and 26). The reaction in the stratum corneum and granulosum followed the same pattern of the previous concentrate-fed groups. In the stratum granulosum, PAS-positive, diastase-resistant droplets were more obvious. In the superficial layers of the stratum spinosum, the cytoplasm stained diffusely and contained pink granules. The density of the intercellular PAS- reaction increased in the transitional zone and continued into the stratum corneum. In stratum corneum, the intra-cellular PAS reaction of the superficial horn cells which blended with the intense reaction of the ingesta and bacteria on the surface of the epithelium was more obvious.

Table 10: Effects of the duration of concentrate feeding on intensity of Periodic Acid-Schiff (PAS)–Reaction. (N = 3 animals for each treatment)

Feeding regime	Strratum corneum	Stratum granulosum	Stratum Spinosum (Superficial)	Stratum Spinosum (Deep)
CF0W	+++	+	0	0
CF2T	+++	+	0	0
CF4T	+++	+	0	0
CF1W	+++	++	+	0
CF2W	+++	++	+	0
CF4W	+++	++	++	0
CF6W	+++	++	++	0
CF12W	+++	+	0	0

0= No reaction + = Weak ++ = Moderate +++ = Strong

Cell boundaries of the stratum corneum were thicker in 4 weeks concentrate-fed group compared to 6 weeks concentrate-fed group (Fig. 25 and 26). After 12 weeks of concentrate feeding, the degree of PAS- reaction became weak and is similar to those up to 4 days concentrate-fed group (Fig. 27).

The basement membrane and the fibers of the lamina propria showed a PAS- diastase stable - positive reaction in all treated groups (Fig. 24 a).

Glycogen: Periodic Acid-Schiff-reactive material that was removed by digestion with diastase was assumed to be glycogen. It was not observed in the mucosa of all groups.

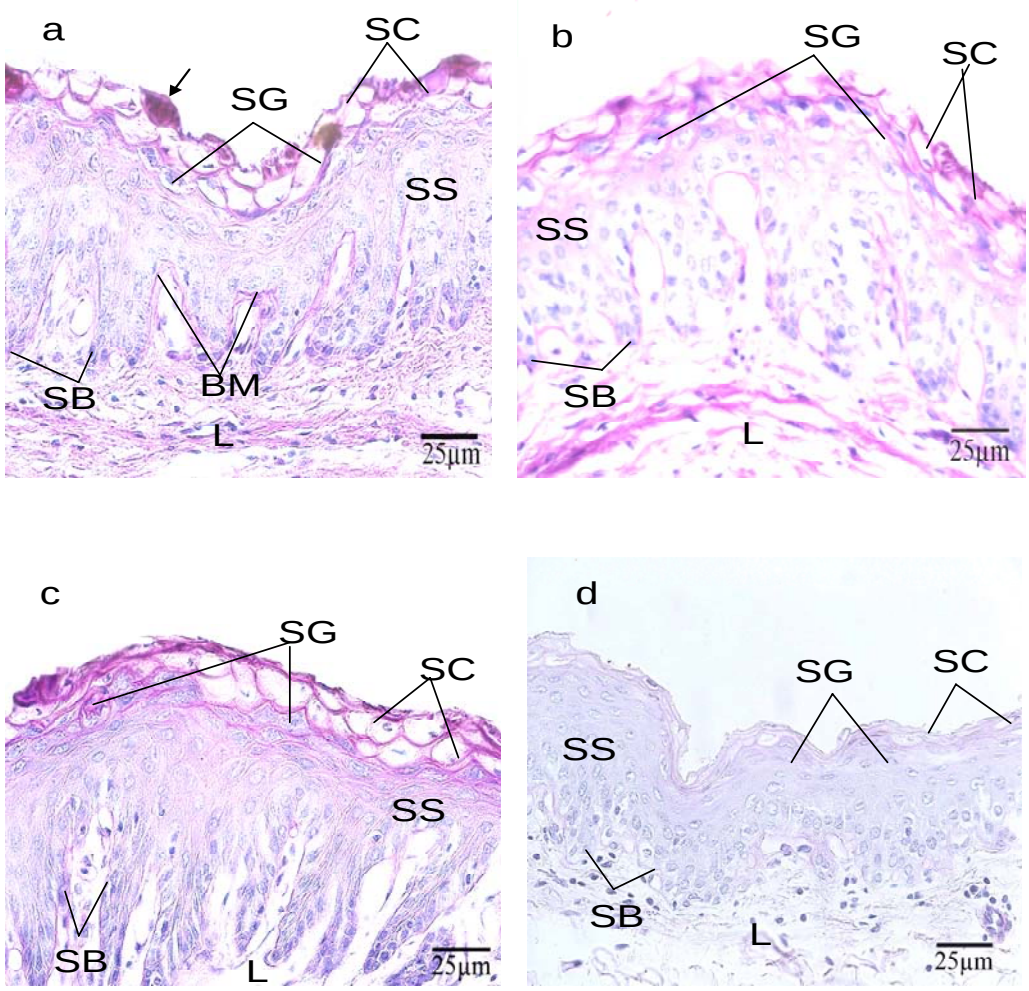

Fig. 23: Paraffin-sections stained by Periodic-Acid Schiff´s method, showing different degree of reaction. Intensely stained stratum corneum (SC) and connective tissue of the lamina propria (L), weakly stained stratum granulosum (SG) and stratum spinosum (SS) compared to negative basal cells (SB). Arrow (→) points to horn cells containing ingesta a. weak PAS-reaction (hay-fed group) b. moderate PAS-reaction (2 weeks concentrate-fed group) c. strong PAS-reaction (4 weeks concentrate-fed group) d. control section-negative. (Scale 25µm)

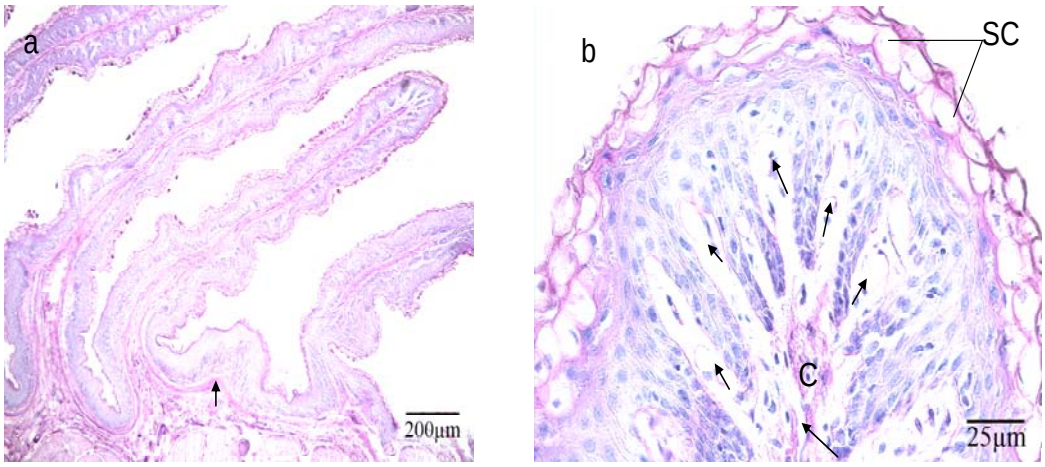

Fig. 24: Paraffin section stained by Periodic-Acid Schiff´s method (hay-fed group), showing a. well stained, condensed lamina propria (arrow) (Scale 200µm)

b. well stained stratum corneum (SC), connective tissue at the core of the papilla (C). Arrows (→) points to the blood capillaries. (Scale 25µm)

3.2.2 Quantitative histological findings

3.2.2.1 The effect of duration of concentrate feeding on the mean thickness of the epithelium and its strata.

3.2.2.1.1 Mean thickness of the epithelium

The mean thickness of the epithelium was obtained from both papillary (tip and base) and inter-papillary epithelium. The mean thickness of the epithelium was increased significantly ($P < 0.05$) with the intake of concentrate diet for 4 days to12 weeks (Diagram. 2). Among concentrate-fed groups, thickness of the epithelium increased gradually with increasing the duration of feeding up to the 6 weeks, however, differences were only significantly ($P < 0.05$) in 2 weeks concentrate-fed group compared to 2 days concentrate-fed group and in 6 weeks concentrate-fed group compared to all concentrate-fed groups (maximum value). In 12 weeks concentrate-fed group, the thickness of the epithelium was decreased significantly ($P < 0.05$) from that of 6 weeks concentrate-fed group.

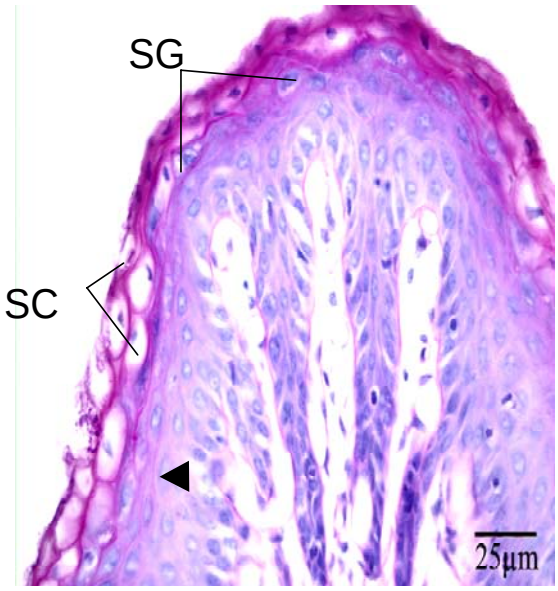

Fig. 25: Paraffin-section of a rumen papilla (4 weeks concentrate-fed group). Periodic-Acid Schiff-(PAS)-positive, diastase-resistant material was present in thick accumulations in the intercellular spaces, within the horn cells (SC) and granular cells (SG). Arrow (→) pointed to PAS-positive reaction within some superficial spiny cells (Scale 25µm)

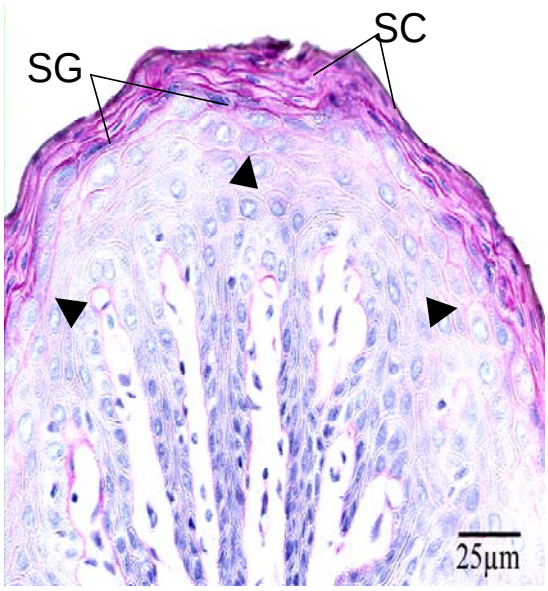

Fig. 26: Paraffin-section of a rumen papilla (6 weeks concentrate-fed group). Periodic-Acid Schiff-positive (PAS), diastase-resistant material was present in thick accumulations in the intercellular spaces, within the flattened horn cells (SC) and granular cells (SG). PAS-positive reaction within superficial spinosal cells was more obvious (arrow) (Scale 25µm).

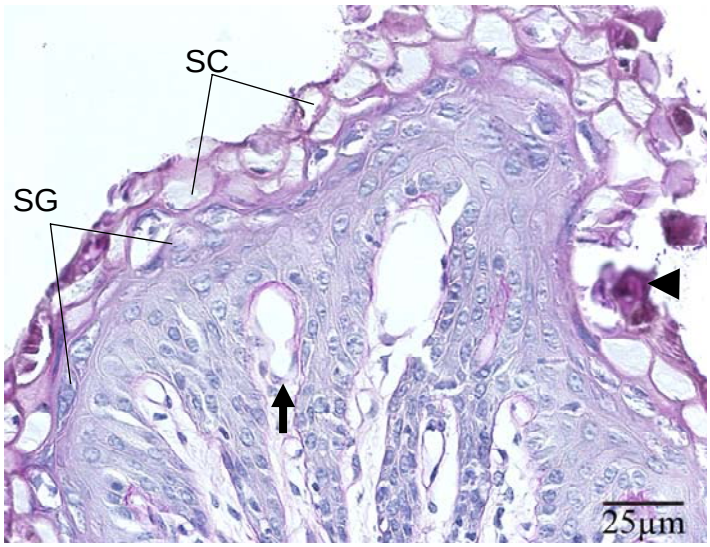

Fig. 27: Paraffin-section of the rumen papilla (12 weeks concentrate-fed group), shows weak PAS-positive reaction within both horn cells (SC) and granular cells (SG). Arrow (→) points to sinusoid-like capillary. Notice: horn cells containing ingesta (arrow head). (Scale 25µm)

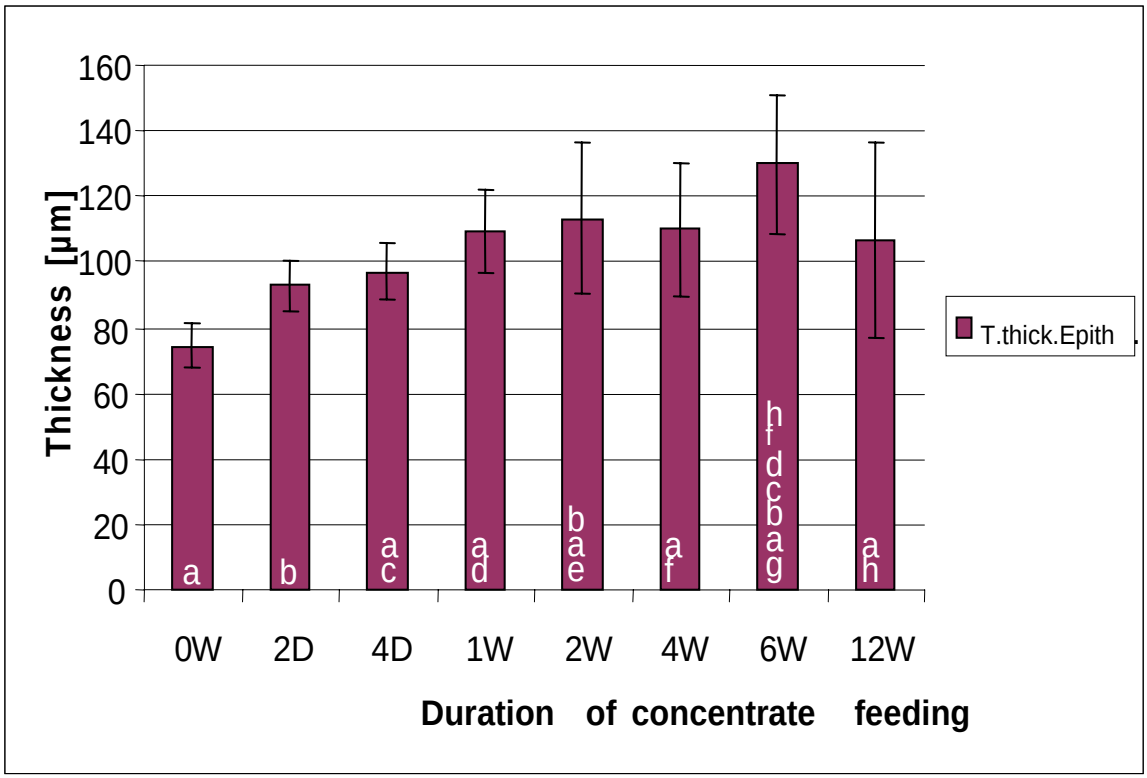

Diagram 2: Effects of the duration of concentrate feeding on the total thickness of the epithelium (Mean±SD). a-h Means in the different columns and kind of sampling sharing the same superscript letters differ significantly.

3.2.2.1.2 Mean thickness of the strata germinativum (basale + spinosum) and granulosum (living cell layers of the epithelium)

The mean thickness of the stratum germinativum which includes the stratum basale and spinosum and the stratum granuolosum was obtained from those comprised both papillary (tip and base) and inter-papillary epithelium. The mean thickness of living cell layers of the epithelium was increased significantly ($P < 0.05$) in concentrate-fed groups compared to that of the hay-fed group. However, in 4 and 12 weeks concentrate-fed group, differences were not significance (Diagram. 3). Concentrate feeding up to 2 weeks and in 6 weeks resulted in a gradual increase in the thickness of the living cell layers of the epithelium. It decreased in 4 weeks and 12 weeks. However, differences were not significance. Concentrate feeding for 6 weeks resulted in a significant increase ($P < 0.05$) of the living cell layers of the epithelium compared to that of 4 or 12 weeks. Concentrate feeding for 12 weeks resulted in a remarkable decrease of the thickness of the living cell layers of the epithelium from that of all concentrate-fed groups. However, the difference was only significant from 1 week and 6 weeks concentrate-fed group.

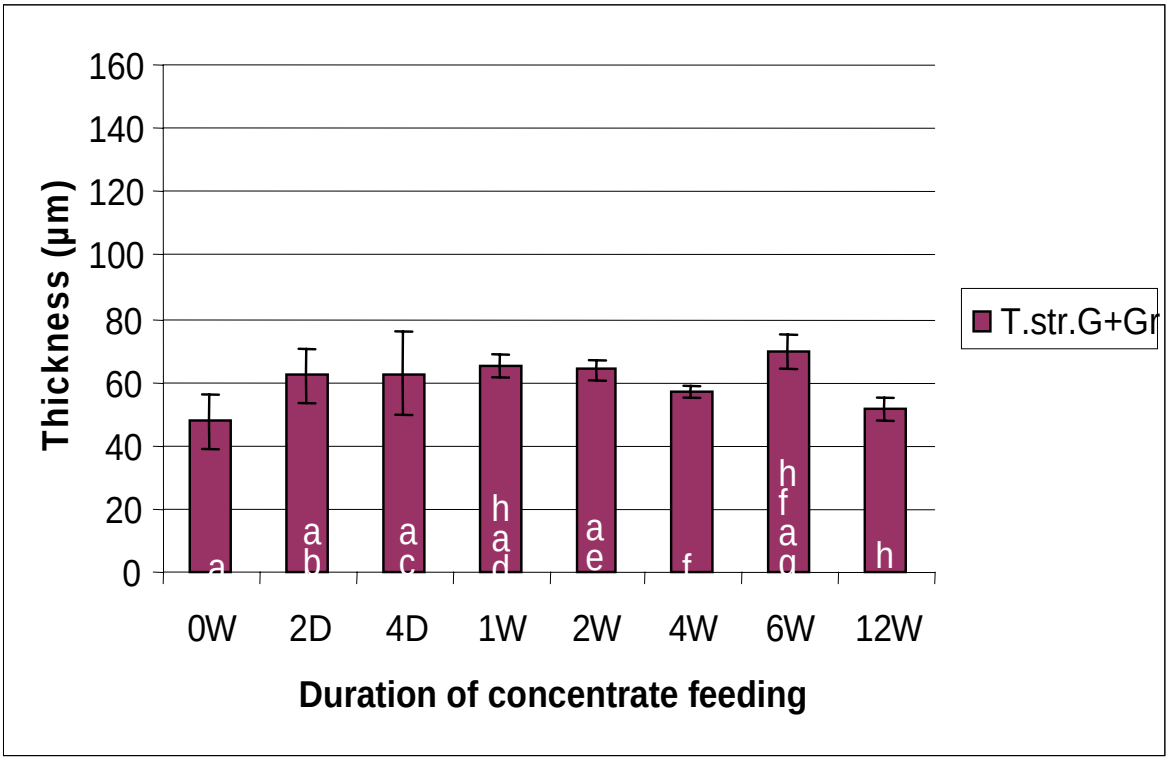

Diagram 3: Effects of duration of concentrate feeding on the mean thickness of the stratum germinativum and granulosum. a-h Means in the different column and kind of sampling sharing the same superscript letters differ significantly.

3.2.2.1.3 Mean thickness of the stratum corneum

The mean thickness of the stratum corneum (dead cells) of the epithelium was obtained from those comprised both papillary (tip and base) and inter-papillary epithelium. The thickness of the stratum corneum decreased from that of hay fed animals (control group) after 2 or 4 days of concentrate feeding and increase gradually within 1 week (Diagram. 4). However, the differences were not significant. In 2 weeks concentrate fed- group, the thickness of the stratum corneum increased significantly ($P < 0.05$) compared to that of animals fed concentrate for 2 or 4 days. In the 4 weeks concentrate-fed group, the thickness of the stratum corneum increased significantly compared to that of animals fed concentrate for up to 1 week. Concentrate feeding for 6 weeks resulted in a decrease in the thickness of the stratum corneum compared to that of 4 weeks concentrate-fed group. However, the difference was not significant. Concentrate feeding for12 weeks increased significantly ($P < 0.05$) the thickness of the stratum corneum compared to that of control group and all concentrate-fed groups (maximum value).

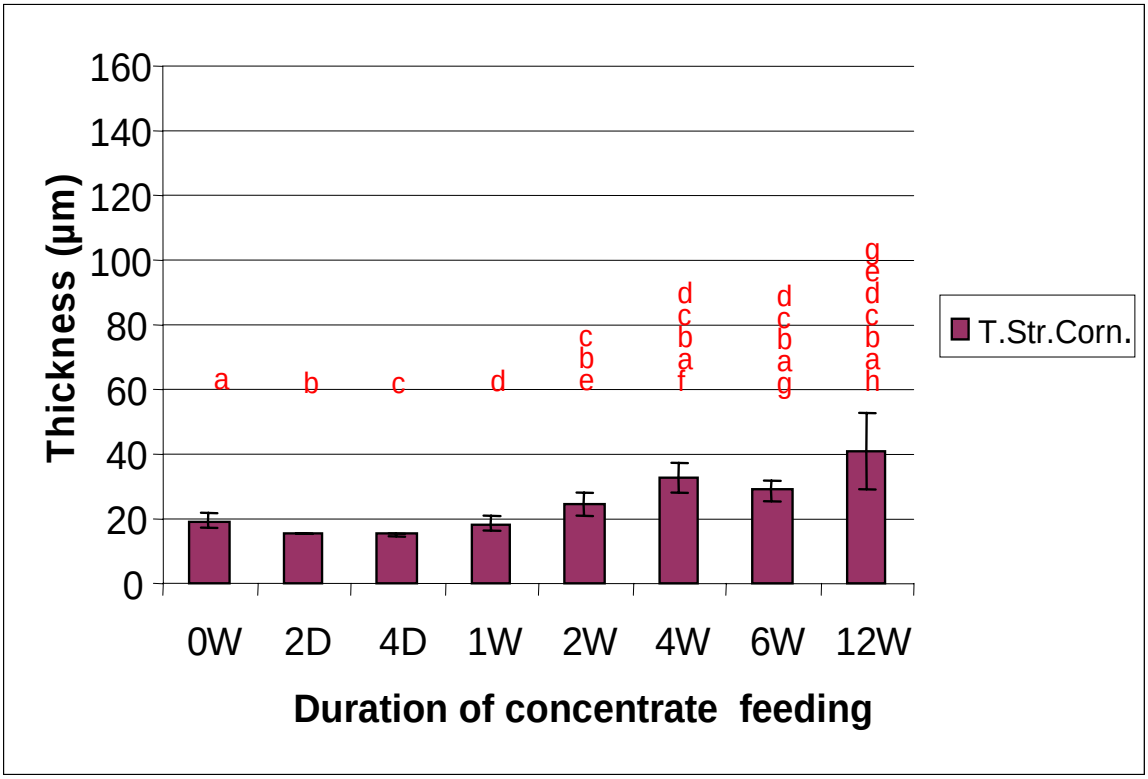

Diagram 4: Effect of duration of concentrate feeding on mean thickness of stratum corneum. a-h Means in the different columns and kind of sampling sharing the same superscript letters differ significantly.

3.2.2.1.4 Relation between strata (germinativum + granulosum) and stratum corneum

Relation between strata (germinativum + granulosum) (living cell layers of the epithelium) and stratum corneum (dead cells) was obtained by dividing the mean thickness of the living cell layers of the epithelium over the mean thickness of the stratum corneum. Generally, it increased significantly in animals fed concentrate for 2-4 days compared to that of the hay-fed group and 2-12 weeks concentrate-fed groups (Diagram. 5). The relation decreased gradually within 1-12 weeks of feeding concentrate, except in 6 weeks concentrate-fed group, where it showed a significant increase ($P < 0.05$) compared to that of 4 and 12 weeks concentrate-fed groups. The relation decreased significantly ($P < 0.05$) in 4 and 12 weeks concentrate-fed groups from that of hay-fed group. There were neither significant differences between animals fed concentrate for 2-12 weeks, nor between 2 and 6 concentrate-fed groups (Diagram. 5).

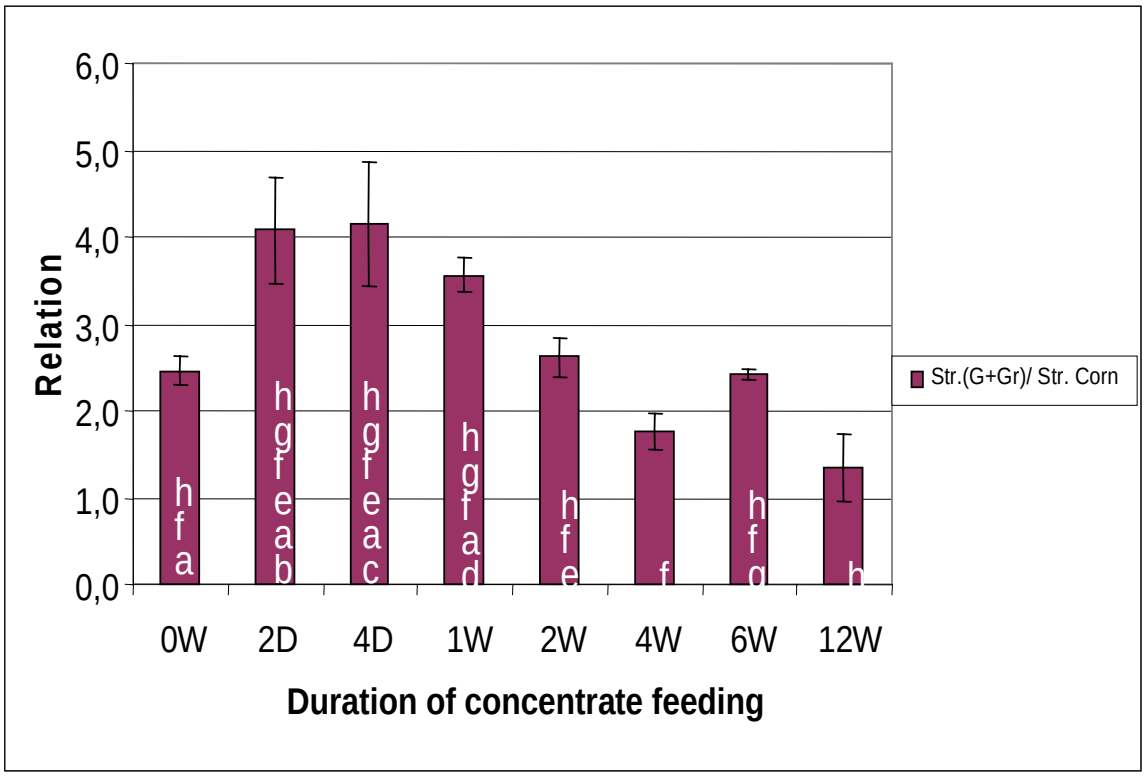

Diagram 5: Effects of the duration of concentrate feeding on the relation between stratum (germinativum + granulosum) and stratum corneum. a-h Means in the different columns and kind of sampling sharing the same superscript letters differ significantly.

3.2.2.2 The effect of duration of concentrate feeding on the thickness of the epithelium and its strata at different locations of the rumen-mucosa (at the tip of the papillae, base of the papillae and inter-papillae regions)

3.2.2.2.1 Thickness of the epithelium

Among the hay-fed group (control-group) and all concentrate-fed groups, variations in the thickness of the epithelium located either on the tip or base of the papillae or on the interpapillary mucosa were observed. Generally, the epithelium covering the papillae was thicker than that covering the interpapillary regions and at the tip of the papillae was thicker than that at the base. The hay-fed group showed a minimum thickness of the epithelium at the different locations compared to those of concentrate-fed groups (Diagram. 6). Concentrate feeding resulted in a gradual increase in the thickness of the epithelium, at the different locations. However, the difference was significant ($P < 0.05$) only in animals fed concentrate for 1-6 weeks compared to that of the hay-fed group. Concentrate feeding for 4 days and 12 weeks resulted in a significant ($P < 0.05$) increase of the thickness of the epithelium at the base of the papillae and that covering the both papillary base and tip, respectively, compared to that of the hay-fed group. Among concentrate groups, there were no significant differences between animals fed concentrate for 2 days, 4 days and 1 week. Concentrate feeding for 2 weeks and 4 weeks resulted in a significant increase ($P < 0.05$) in the thickness of epithelium located on the tip of the papillae compared to that of 2-4 days and 2 days, respectively. Concentrate feeding for 6 weeks resulted in significant increase ($P < 0.05$) (maximum value) in the thickness of the epithelium located on the base of the papillae compared to that of all concentrate-fed groups. However, thickness of epithelium located on the tip of the papillae of 6-12 weeks increased significantly ($P < 0.05$) compared to that of 2-4 days concentrate-fed groups.

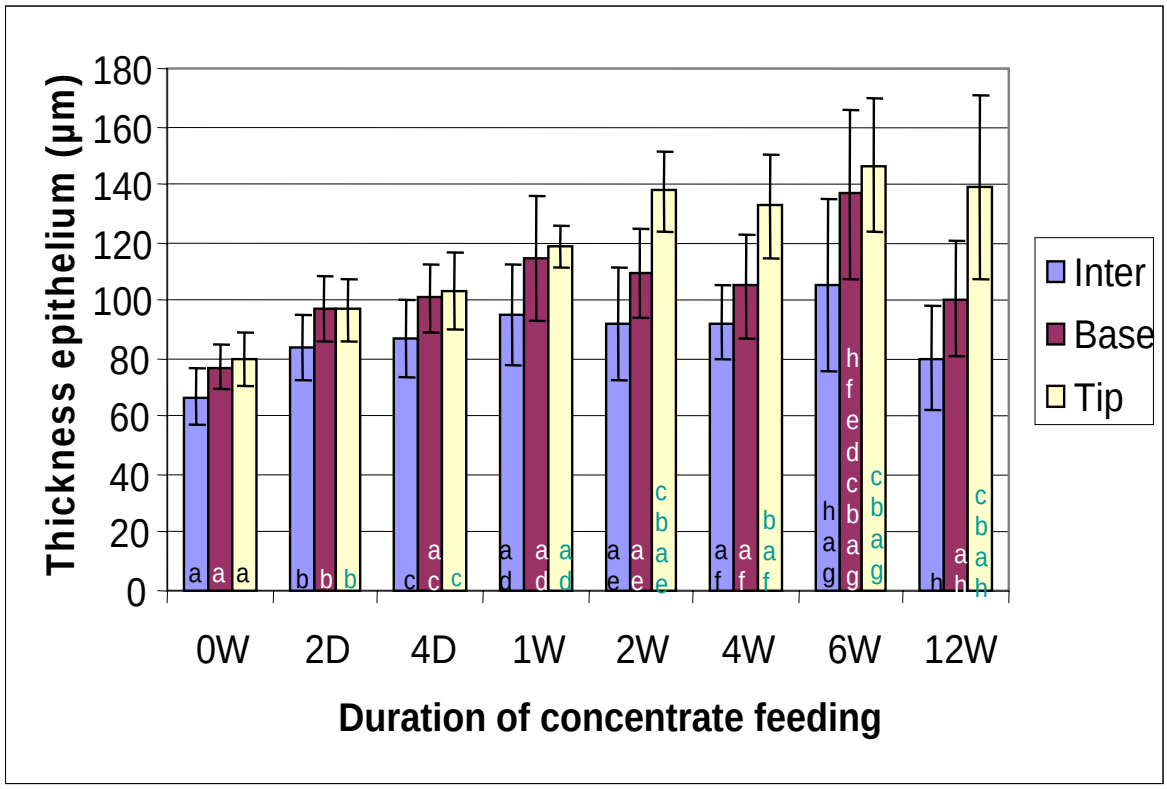

Diagram 6: Effects of duration of concentrate feeding on the thickness of the epithelium at the tip, base and interpapillary regions. a-h Means in the different columns and kind of sampling sharing the same superscript letters differ significantly.

3.2.2.2.2 Thickness of the stratum germinativum and granulosum (living cell layers of the epithelium)

Generally, concentrate feeding resulted in a significant increase ($P<0.05$) in the thickness of living cell layers of the epithelium at the tip (1 week, 2 weeks and 6 weeks) and the interpapillary regions (2 or 4 days, 2 weeks and 6 weeks) compared to those of hay-fed group (control group). There was no significant difference at the level of papillary base between the groups (Diagram. 7). Within concentrate-fed groups, these thicknesses were decreased in 1-4 weeks concentrate-fed groups compared to that of 2-4 days concentrate-fed groups. However, the difference was not significant except between 1 week and 4 days concentrate-fed groups (at the tip of papillae). In 6 weeks concentrate-fed group, thickness was increased compared to that of all concentrate-fed groups. The differences were not significant except for 4 days concentrate-fed group (at the tip of papillae) and 12 weeks concentrate-fed group (at the papillary base and interpapillary regions).

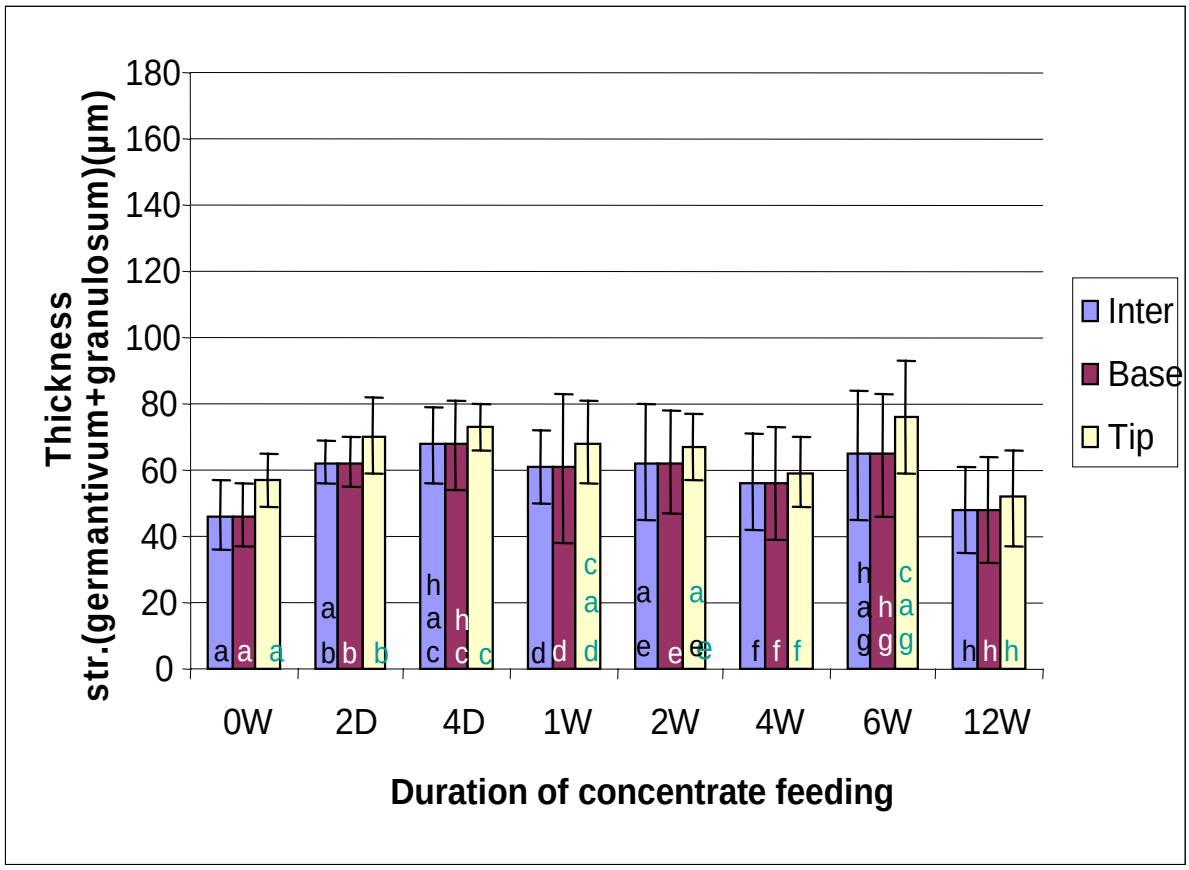

Diagram 7: Effects of duration of concentrate feeding on the thickness of the stratum germinativum and granulosum at the tip, base and interpapillary regions. a-h Means in the different columns and kind of sampling sharing the same superscript letters differ significantly.

In 12 weeks concentrate-fed group, the thickness was decreased compared to that of all concentrate-fed groups. The differences were not significant except for 4 days and 6 weeks concentrate-fed groups.

3.2.2.2.3 Thickness of the stratum corneum

Generally, the thickness of the stratum corneum at the papillary base was higher than that at the tip and interpapillary region. Concentrate feeding for 2 or 4 days resulted in a decrease of the thickness of the stratum corneum compared to that of control group. The difference was not significant. Concentrate feeding for one week or 2 weeks resulted in an increase in the thickness of stratum corneum compared to those fed concentrate-diet for 2 or 4 days. The difference was not significant. Concentrate feeding for 4-12 weeks resulted in a significant

increase (P<0.05) of the thickness of the epithelium in mentioned locations compared to hay-fed group, except on the papillary base of 4-6 weeks concentrate-fed groups, where the difference was not significant. Moreover, there was a significant difference (P<0.05) between 4-12 weeks concentrate-fed groups and those fed concentrate-diet for up to one week (Diagram. 8). Concentrate feeding for 12 weeks resulted in a maximum increase in the thickness of the stratum corneum compared to those of all concentrate-fed groups. However, this group showed well developed papillary stratum corneum compared to the interpapillary one. Moreover, thickness of the stratum corneum located at the base of papillae increased significantly (P<0.05) compared to that of animals fed concentrate for up to 2 weeks. Thickness of the stratum corneum located at the tip of the papillae increased significantly (P<0.05) compared to that of animals fed concentrate for up to 6 weeks.

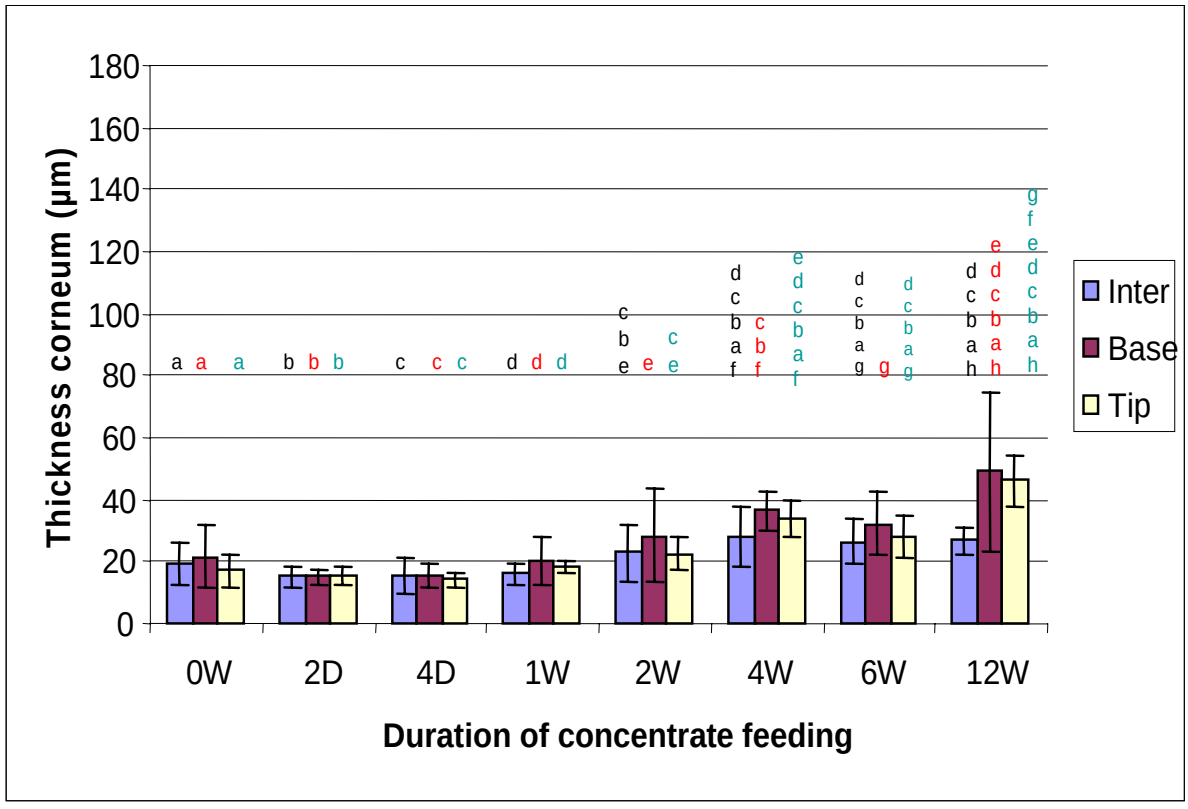

Diagram 8: Effects of duration of concentrate feeding on the thickness of the stratum corneum at the tip, base and interpapillary regions. a-h Means in the different columns and kind of sampling sharing the same superscript letters differ significantly.

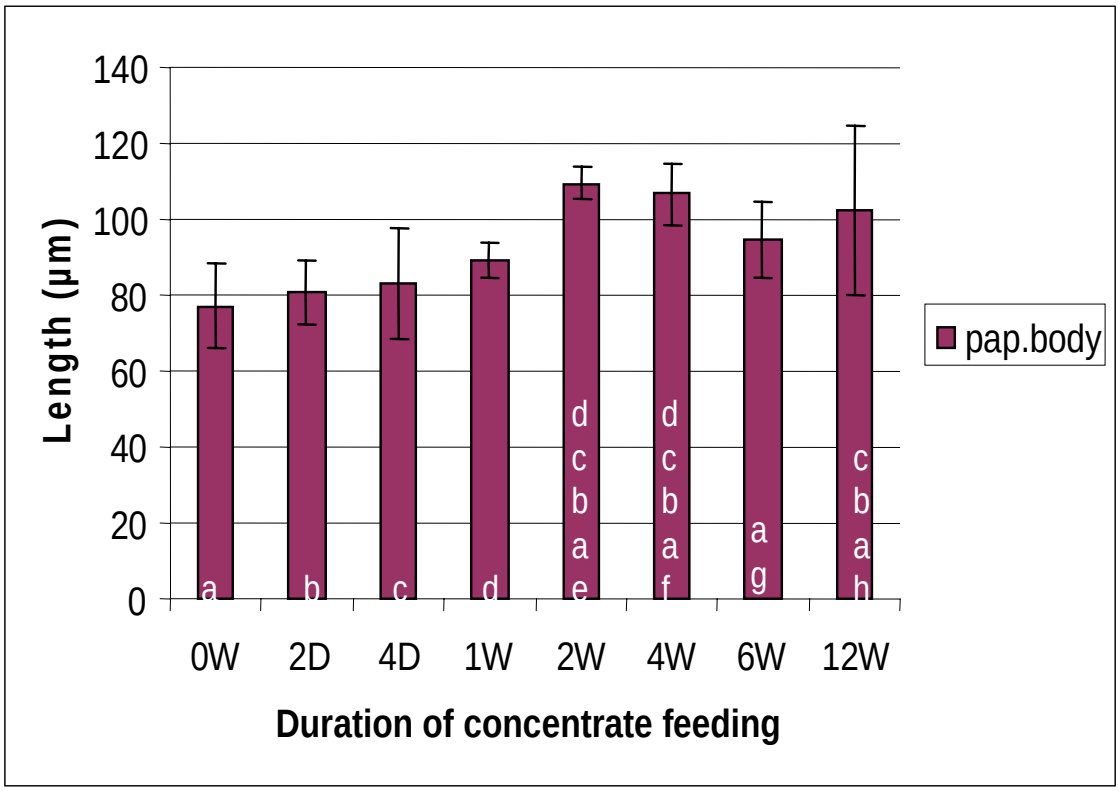

Diagram 9: Effects of duration of concentrate feeding on the length of the papillary body. a-h Means in the different columns and kind of sampling sharing the same superscript letters differ significantly.

In for 2-12 weeks concentrate-fed groups, the mean length of the papillary body was increased significantly ($P < 0.05$) compared to that of hay-fed group. Within concentrate-fed groups, the mean length of the papillary body increased significantly ($P < 0.05$) in 2-4 weeks concentrate-fed groups compared to that of animals fed concentrate for up to 1 week (Diagram. 9). The mean length of the papillary body reached its maximum value in 2 weeks concentrate-fed group. In 6 weeks concentrate-fed group, there was a decrease in the mean length of papillary body compared to 2 weeks concentrate-fed group. However, the differences were not significant. Concentrate feeding for 12 weeks resulted in an increase in the mean length of the papillary body compared to that of 6 weeks concentrate-fed group. It showed significant differences compared to those fed concentrate-diet for 2 or 4 days.

3.2.2.2.4 Effects of concentrate feeding on the length of the papillary body in the region of the tip of the papillae.

The papillary body was developed at the tip of the papillae than at the base of the papillae or at the intrpapillary regions, respectively. Generally, the mean length of the papillary body was increased with the intake of concentrate feeding for different intervals of time (Diagram. 9).

3.3 Immunohistochemical findings of the sheep rumen

3.3.1 Immunohistochemical evaluation of the muscularis mucosa in the sheep rumen (αS.M.A)

Muscularis mucosa was not detected in the rumen of all sheep groups. Instead, numerous cells showing constantly intense immunoreactivity for α SMA were observed in the core of ruminal papillae and interpapillary mucosa near the epithelium (Fig. 28). These cells established a layer, the condensed fibrous layer, at the position equivalent to the muscularis mucosa. This layer showed two-dimensional extension parallel to the epithelium and extended following the contour of the ruminal papillae. Additionally, more abundant actin-immunoreactive cells were detected in the ruminal papillae than the interpapillary mucosae (Fig. 28).

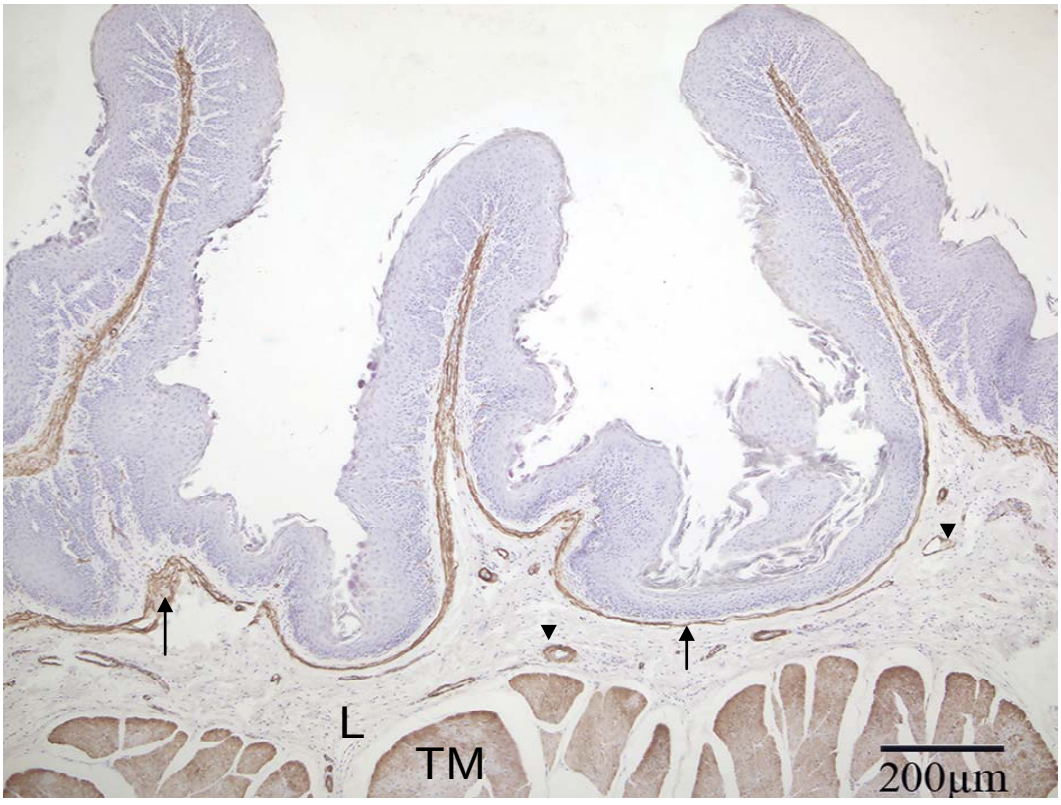

Fig. 28: Micrograph of the rumen of the sheep fed concentrate for one week. Tunica muscularis (TM), vascular wall (arrow heads) and condensed fibrous layer (arrows) showed immunoreactivity for α-smooth muscle actin. L: lamina propria. (Scale 200µm)

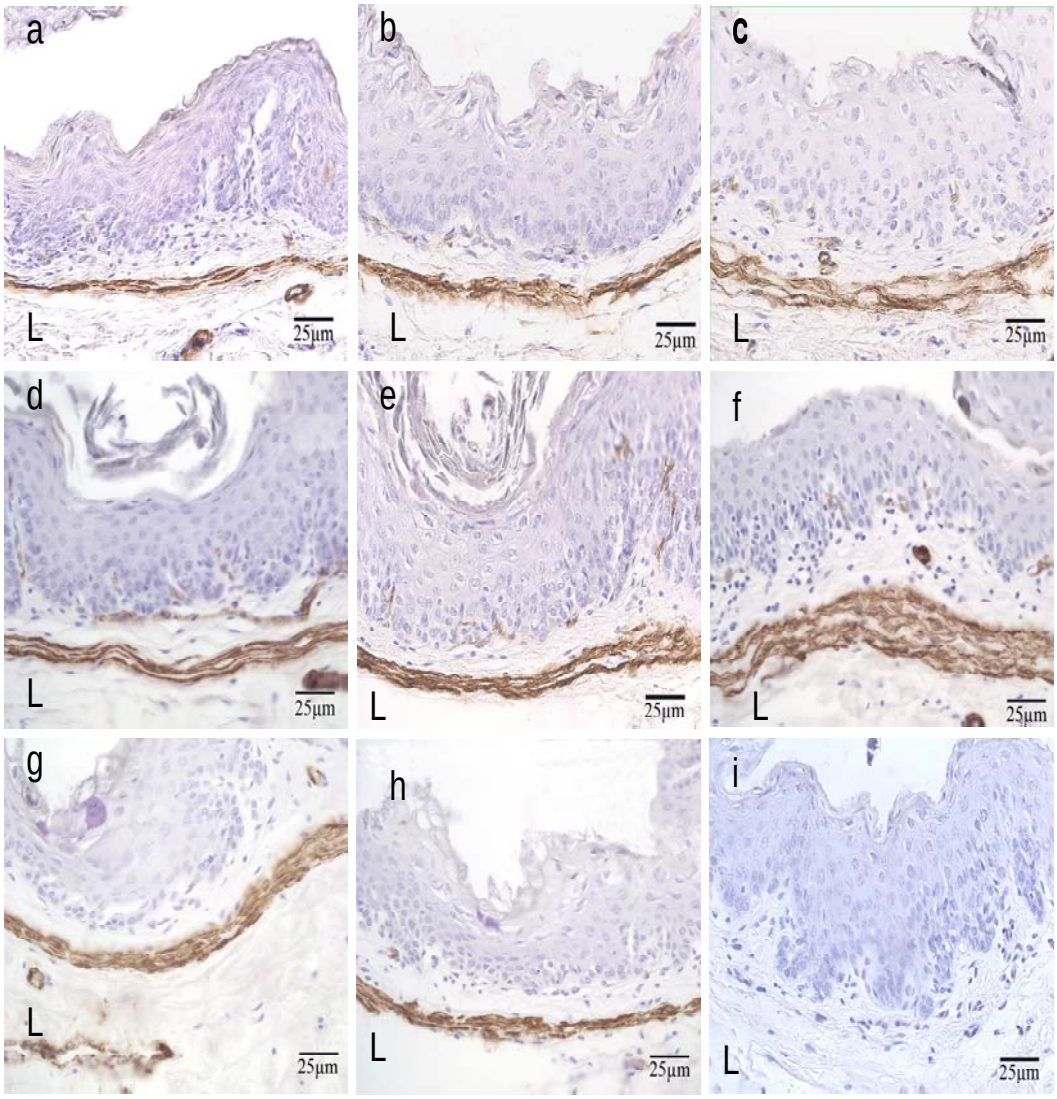

Fig. 29: Micrographs of the rumen of a. Hay-fed group, b. 2 days concentrate-fed group, c. 4 days concentrate-fed group, d. one week concentrate-fed group, e. 2 weeks concentrate-fed group, f. 4 weeks concentrate-fed group, g. 6 weeks concentrate-fed group and h. 12 weeks concentrate-fed group. i. Negative control. Notice the different thickness of the condensed fibrous layer (immunostained for α-smooth muscle actin). (Scale 25µm)

All sheep groups exhibited small abundance of the condensed fibrous layer, but quantitative variations among different concentrate-fed groups were present (Fig. 29). The condensed fibrous layer was very thick in 4 weeks concentrate-fed group, thick in 2 and 6 weeks concentrate-fed group, thin in hay-fed group and 2-4 days, 1 week and 12 weeks concentrate-fed groups

Blood vessels having muscular wall were rare in the propria mucosa between the condensed fibrous layer and the epithelium. They were more obvious at the submucosa formed the core

of the papillae, especially at the base. In 4 weeks concentrate-fed group, large density of blood vessels was observed comparing to hay-fed group (Fig. 30).

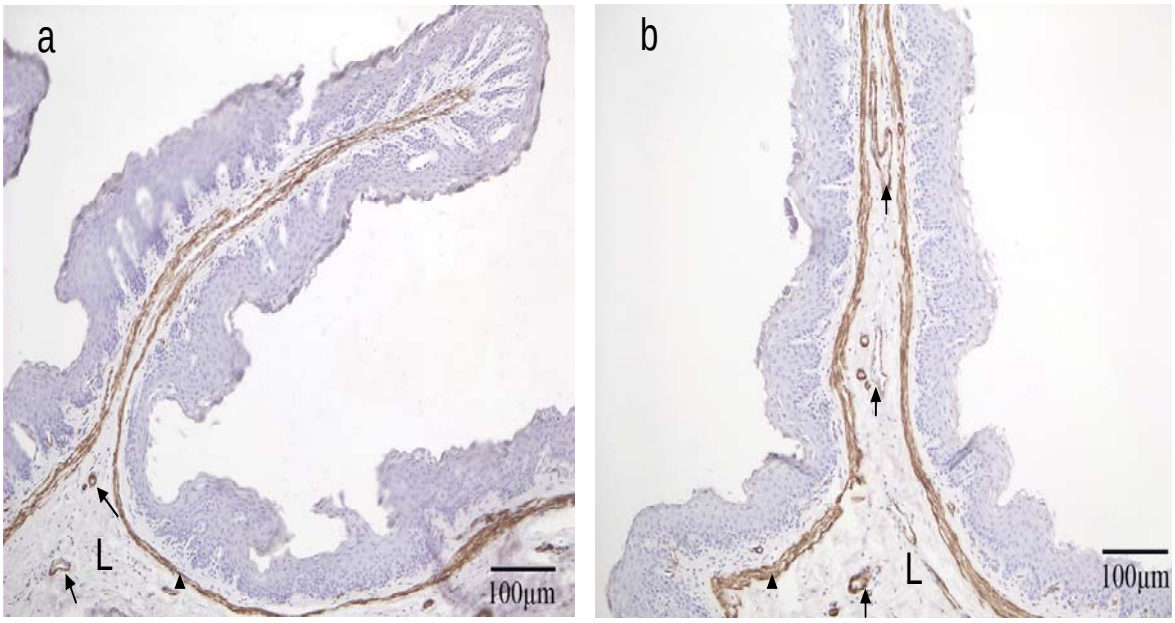

Fig. 30: Micrographs of the ruminal papilla from a. hay-fed group (control), b. 4 weeks concentrate-fed group, showed immunoreactivity of both condensed fibrous layer (arrow heads) and vascular wall (arrows) for α-smooth muscle actin. Notice the large numbers of the blood vessels located at the papillary core of 4 weeks concentrate-fed group. L: lamina propria. (Scale 100µm)

3.3.2 Immunolocalization of Na^+ / H+ exchanger isoforms (NHE1 and NHE3) in the rumen epithelium

NHE3 transporter protein isoform was localized at both apical plasma membrane (more dense) and intracellular locations (Table. 11; Fig. 31). NHE1 isoform was not detected in the sheep-rumen epithelium. NHE3 was found to be more intense at both stratum granulosum and stratum spinosum (superficial layer adjacent to stratum granulosum), with decreasing intensity through stratum spinosum (deep or suprabasal layer) and stratum basale. Stratum corneum was negative (Fig. 31). Distribution of NHE3 isoform was different within the different strata. In stratum granulosum, NHE3 isoform was presented at apical and basolateral cell membrane. In stratum spinosum (superficial layer), NHE3 isoform was distributed

predominant at the apical surface / membrane of the cells. Meanwhile, in both stratum spinosum (deep layer) and stratum basale, intracellular NHE3 isoform was predominantly (Fig. 31).

Table 11: The effect of type and duration of concentrate feeding on the expression of NHE3 at different strata of the rumen epithelium. (N = 3 animals for each treatment)

Animals	Str. granulosum	Str. spinosum (superficial)	Str. spinosum (deep or suprabasal layer)	Str. basale
CF0W	++	++	+	+ / 0
CF2 D	++	++	+	+ / 0
CF4 D	++	++	+	+ / 0
CF1W	+	++	+	+ / 0
CF2W	+++	+++	+	+
CF4W	++++	++++	+++	++
CF6W	++	++	+	+
CF12W	++	++	+/ 0	+/ 0

0= no reaction += very weak ++= weak +++= moderate ++++= strong

Immunohistochemical staining for NHE3 in rumen epithelium showed different degrees of staining among sheep groups (Table. 11). Rumen epithelium of hay-fed group, 2-4 days and 1 week concentrate-fed groups showed weak antibody reactions. Positive NHE3-immunostaining was observed at granular cells and superficial spiny cells (apical surface) and decreased in intensity through stratum spinosum (suprabasal layer) to stratum basale (Fig. 31). Rumen epithelium from 2 weeks concentrate-fed group showed moderate antibody reactions. Rumen epithelium from 4 weeks concentrate-fed group, showed strong antibody reactions compared to the previous mentioned groups (Fig. 31).

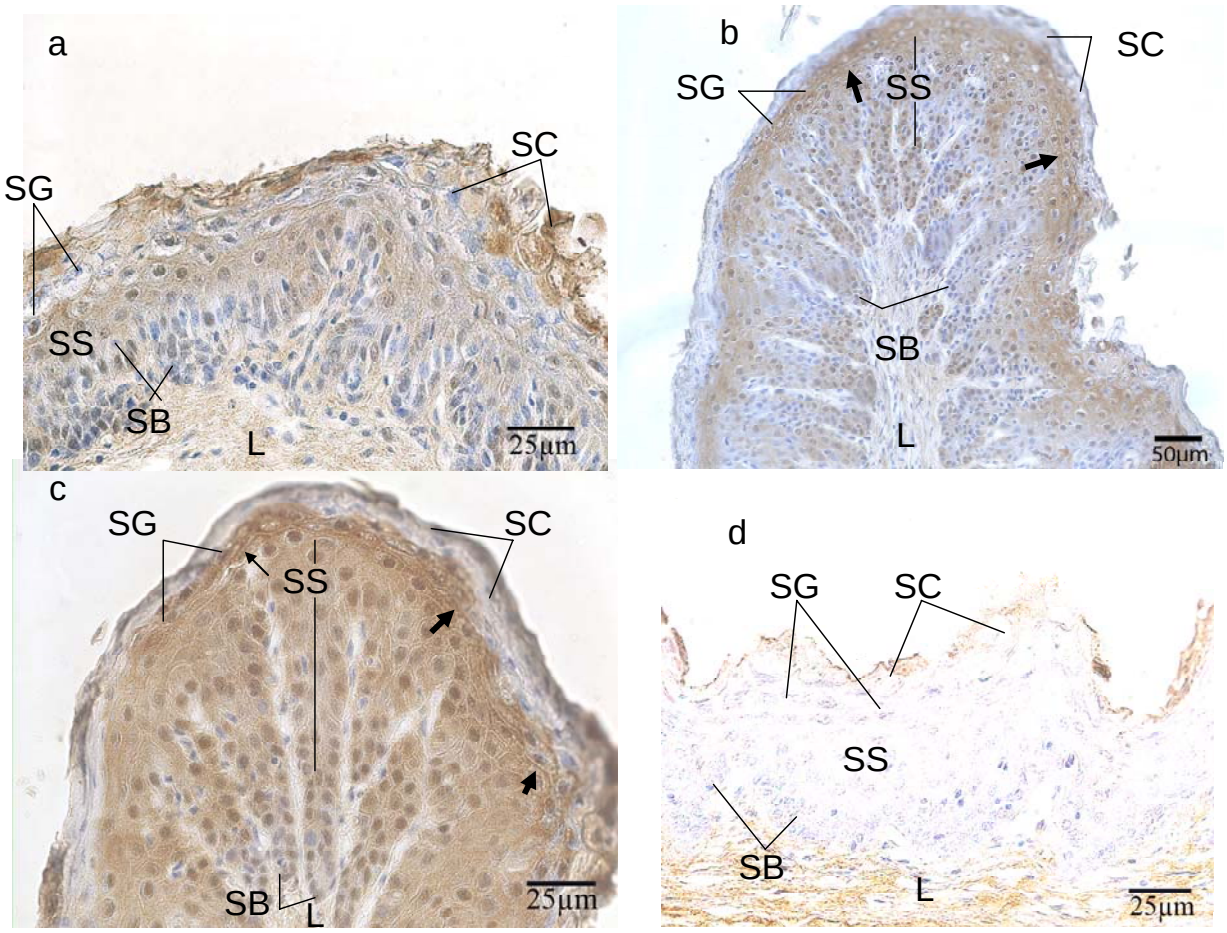

Fig. 31: Micrographs of the rumen epithelium showing immunoreactivity for NHE3 staining among sheep groups a. Hay-fed group (weak-reaction) b. 2 weeks concentrate-fed group (moderate-reaction) (Scale 50µm) c. 4 weeks concentrate-fed group (strong-reaction) d. Negative control. Notice: the intense staining of the NHE3 at both deep layer of stratum granulosum (SG) and superficial layer of stratum spinosum (SS), with decreasing intensity through the suprabasal spiny cells and cells of stratum basale (SB). Stratum corneum (SC) was negative. Arrows (→) pointed to the apical staining of the NHE3. L: lamina propria. (Scale 25µm)

The intense staining occurred in all strata of the epithelium except stratum corneum, however, it was more intense at granular cells (at apical and basolateral membrane) and superficial spiny cells (at apical surface). Also, some basal cells showed apical staining for NHE3 isoform. Rumen epithelium from 6 or 12 weeks concentrate-fed groups showed weak and very weak antibody reactions, respectively. Weak local reaction for NHE3 isoform was seen at both stratum granulosum and stratum spinosum (superficial layer adjacent to stratum granulosum), with decreasing intensity throughout stratum spinosum (suprabasal layer) and stratum basale (Fig. 31).

3.3.3 Gap-junctions (Connexin 43)

Cell borders of the rumen epithelium have different affinity to connexin 43 immunostaining (Cx43). Plasma membrane connexin 43 immunostaining was most intense at the stratum spinosum (suprabasal layer) and stratum basale, decreased in intensity throughout stratum spinosum (superficial layers) to stratum granulosum. Stratum corneum was negative (Table 12).

Immunostaining for connexin 43 was found peripherally around the cells and was stronger in the apical membranes (fig. 32). Moreover, the degree of the antibody-reaction differed between hay fed group and concentrate-fed groups (table. 34). Rumen epithelium of hay fed group presented very weak to weak degree of reaction. However, the degree of the antibody-reaction within concentrate-fed groups was weak (2 days concentrate-fed group), moderate (4 days and 1 week concentrate-fed groups), strong (2 weeks concentrate-fed group) and very strong (4, 6 and 12 weeksconcentrate-fed groups) (Fig.33; Table. 12).

Table 12: Effect of type and duration of concentrate feeding on the expression of Cx43 at different strata of the rumen epithelium. (N = 3 animals for each treatment)

Animals	Stratum granulosum	Stratum spinosum (superficial)	Stratum spinosum (suprabasal layer)	Stratum basale
CF0W	+	++	++	++
CF2 D	+	++	++	++
CF4 D	+	++	+++	+++
CF1W	+	++	+++	+++
CF2W	++	+++	++++	++++
CF4W	+++	+++	++++	++++
CF6W	+++	+++	++++	++++
CF12W	+++	+++	++++	++++

0= no reaction += very weak ++= weak +++= moderate ++++= strong

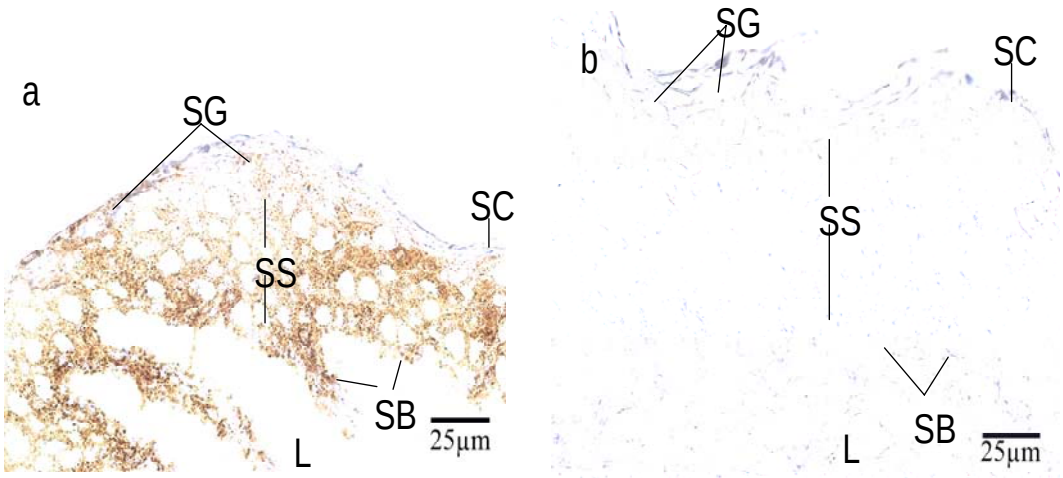

Fig. 32: Micrographs of the rumen epithelium and lamina propria (L) a. Peripheral localization of connexin 43 in the cells of stratum basale (SB) and deep layers of stratum spinosum (SS) with decreasing intensity through the superficial spiny cells and stratum granulosum (SG). Stratum corneum was negative b. Negative control. (Scale 25µm)

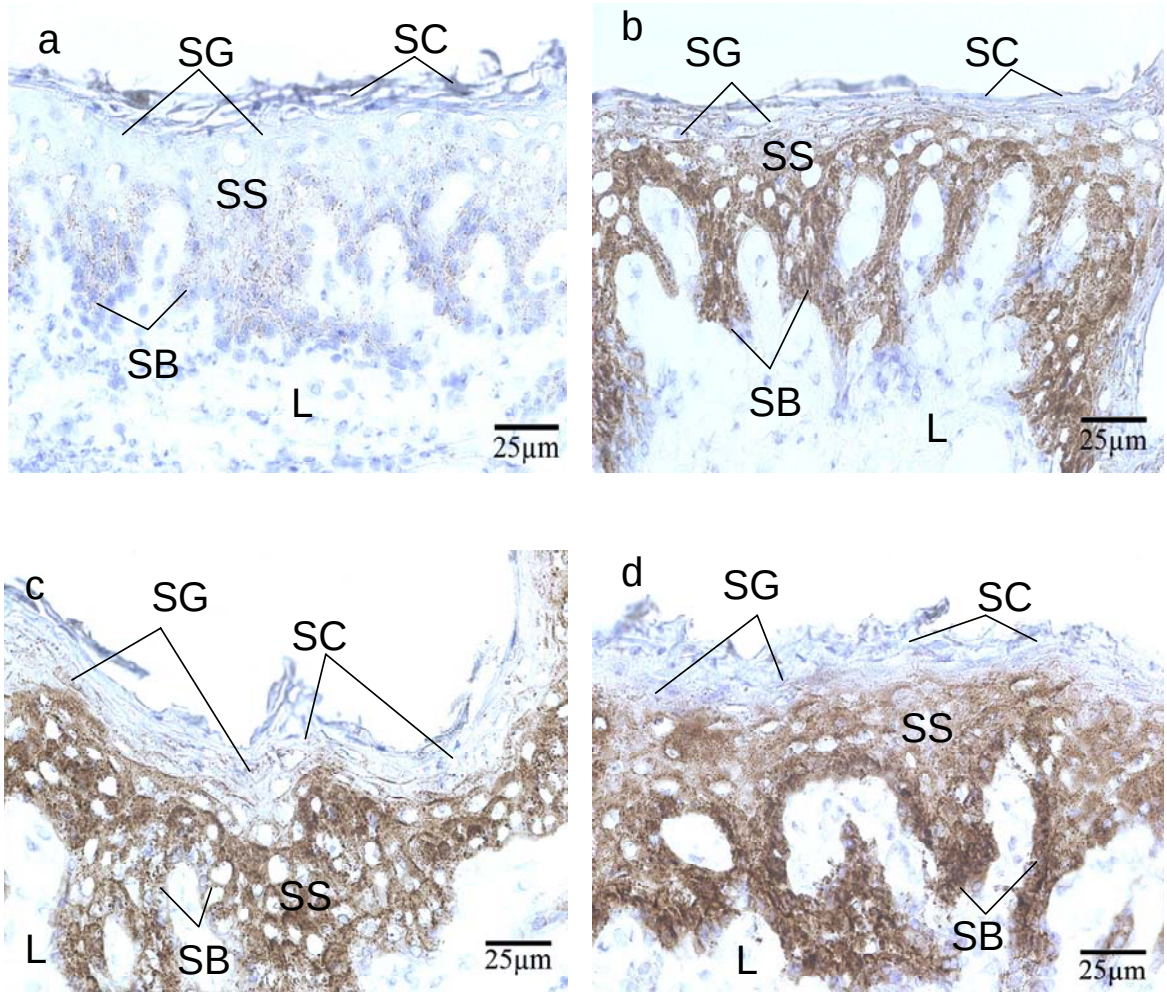

Fig. 33: Micrographs of the rumen epithelium showing different immunoreactivity for connexin 43 among sheep groups: a. very weak-reaction (hay-fed group) b. moderate reaction (one week concentrate-fed group) c. strong reaction (2 weeks concentrate-fed group) d. very strong reaction (4 weeks concentrate-fed group). (Scale 25µm)